USE OF ALTERNATIVE SANITIZERS ON MINIMALLY PROCESSED WATERCRESS HARVESTED IN TWO DIFFERENT SEASONS

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ABSTRACT

There is an increasing concern about the formation of halogenated compounds when sodium hypochlorite (SH) is used as food sanitizer. This research evaluated the quality of watercress harvested in two seasons treated with alternative sanitizers combined with modified atmosphere packaging. Chlorine dioxide (5–10 mg/L), acidified sodium chlorite (250–500 mg/L), peroxyacetic acid (50–90 mg/L) and SH (100 mg/L) were used. Initial respiration rate decreased from 80–135 to 40–72 mg CO_2 kg/h in spring- and summer-harvested watercress. Chlorine dioxide and SH caused a reduction in aerobic mesophilic bacteria of 1.8 and 1.3 log colony-forming unit (cfu/g), respectively. *Enterobacteriaceae* reductions of 1.1 log cfu/g were achieved using SH and peroxyacetic acid in spring and 1.4 log cfu/g by applying acidified sodium chlorite in summer. None of the sanitizers could handle high initial microbial loads for more than 8 days, showing that a raw material with low initial microbial count is required to guarantee the product safety.

PRACTICAL APPLICATIONS

Nowadays, the food market is challenging an increasing demand for safer products that must be elaborated under strict food and environmental standards. In this sense, the application of nontraditional sanitizers could be an alternative to sodium hypochlorite that is being questioned due to the potential formation of halogenated compounds during sanitation. The use of alternative sanitizers in high doses represents a feasible choice for minimally processed vegetables because this technology does not leave toxic residues in foods and neither requires additional investment on industrial facilities, preserves the sensory attributes of vegetables and helps to improve the microbiological quality of ready-to-eat products.

INTRODUCTION

Minimally processed salads, particularly of nontraditional vegetables such as watercress, have increasingly been perceived as foods that contribute to healthy eating habits by consuming vegetables with important input of fibers, vitamins, minerals and bioactive compounds that could prevent some chronic diseases. One of the newly and highly consumed vegetables is watercress (*Nasturtium officinale* R. Br.), which is an aquatic perennial herb belonging to *Brassicacea* family that can be eaten fresh as a salad or blanched and consumed as a regular-processed vegetable. It contains large amounts of sulfur and calcium that influence its characteristic aroma and also provides functional benefits due to its content on vitamin C, provitamin A, folic acid, iodine and iron (Gonçalves *et al*. 2009).

The demand of minimally processed food with high functional value that preserves its freshness attributes has promoted the development of this kind of salads. However, manipulation of vegetables causes physiological stress and wounds, resulting in increased respiration rate and ethylene production, membrane deterioration, water loss and susceptibility to microbial contamination (Artés *et al.* 2007; Escalona *et al.* 2010; López-Gálvez *et al.* 2013).

Disinfection is one of the most critical steps in the development of minimally processed vegetables affecting the quality, safety and shelf life of the final product (Gil *et al.* 2009). The industry has widely used sodium hypochlorite (SH) as a sanitizer due to its high effectiveness to inactivate microorganisms (Francis *et al.* 2012; Gómez-López *et al.* 2013). However, it generates environmental and health risks associated with the formation of carcinogenic halogenated compounds. Nowadays, new environmentally friendly sanitizers are being investigated (Ölmez and Kretzschmar 2009); also, the importance of food without additives is requested.

Peroxyacetic acid (PAA) is known as a strong oxidant that shows antimicrobial action against a broad range of foodborne microorganisms, keeping its activity in a wide range of pH (3.0–8.0) and temperature (5.0–8.0C). Spontaneous decomposition produces harmless compounds such as acetic acid, water and oxygen and it is little influenced by organic material (Beuchat *et al.* 2004; Vandekinderen *et al.* 2009). Targets for the antimicrobial action of PAA include damage to DNA and lipids in the cell membrane, denaturalization of proteins and enzymes and an increase in cell wall permeability by oxidizing sulfhydryl groups and disulfide bonds (Hilgren *et al.* 2007).

Acidified sodium chlorite (ASC) is obtained by lowering the pH of sodium chlorite solution ($NaClO_2$) with any acid generally recognized as safe. It can be used on raw agricultural commodities at chlorite concentrations of 500–1,200 mg/L (pH of 2.3–2.9) (FDA 2010). ASC has been effective on the inactivation of pathogens like *Escherichia coli* O157:H7 and *Salmonella* (Ruiz-Cruz *et al.* 2007).

Chlorine dioxide (ClO_2, CD) is a powerful oxidizing agent with a strong biocide efficacy. It is less affected by low pH and the presence of organic matter, and it is inert toward ammonia to form toxic chloramines or trihalomethanes. The oxidation capacity is 2.5 times higher than SH (Beuchat *et al.* 2005). The effect of ClO_2 was related to nonspecific oxidative damage of the outer membrane leading to the destruction of the transmembrane ionic gradient and loss of permeability control (Gómez-López *et al.* 2009).

According to Mercanoglu-Taban and Halkman (2011), minimally processed lettuces and spinach could be a potential microbiological risk when they are cultivated with untreated irrigation water and inappropriate organic fertilizers. These vegetables can also experience bacterial contamination after harvesting, handling, processing and packaging. López-Gálvez *et al.* (2013) after testing different sanitizers such as H_2O_2, PAA and lactic acid did not report a better control of the microbial growth in minimally processed lettuce compared with tap water. These authors concluded that this kind of sanitation treatments cannot be enough to guarantee the microbial safety of minimally processed vegetables. For that reason, it must be taken extreme care precautions in order to reduce the contamination risk of minimally processed leafy vegetable from farm to fork.

The aim of the current research was to evaluate the overall quality and shelf life of watercress treated with three sanitizers (chlorine dioxide, ASC and PAA), compared with SH as a conventional industry sanitizer, and stored under passive modified atmosphere packaging during cold storage.

MATERIALS AND METHODS

Plant Material

Watercress (*N. officinale* R. Br.) leaves from a hydroponic crop were obtained from a commercial farmer located in the Lonquén area (Más Vida S.A., Santiago, Chile). The watercress harvest was performed in two different seasons. Early spring watercress was harvested in September and summer leaves were harvested 18 weeks later (January). Leaves were transported in a portable ice box at 5C to the laboratory of Centro de Estudios Postcosecha, Facultad de Ciencias Agronómicas of Universidad de Chile (33°34′07.5″S 70°37′49.1″W). Upon arrival watercress leaves were stored overnight in darkness at 0C and 95% relative humidity. The following day, processing was conducted in a disinfected cold room at 8C. Leaves with visual defects, damage or physical decay such as uncharacteristic color (yellow or other) were discarded.

Treatments and Decontamination Procedure

The first experiment (spring-harvested watercress) was conducted according to the conditions showed in Table 1. Sanitizing solutions were prepared using the following reagents: PAA (Tsunami100, Ecolab, St. Paul, MN), CD (Winzaclor-5, Winkler, Santiago, Chile), ASC (Sigma-Aldrich, St. Louis, MO), which was acidified with anhydrous citric acid

TABLE 1. TREATMENTS: DESCRIPTION OF SANITIZING SOLUTIONS AND ATMOSPHERES

Sanitizer	Concentration (mg/L)	Atmosphere	pH	Free Cl_2 (mg/L)
SH	100	PB	6.2	97
SH	100	MAP	6.3	97
CD	10	MAP	7.5	18
CD	5	MAP	7.9	9
ASC	250	MAP	2.8	220
ASC	500	MAP	2.7	500
PAA	50	MAP	4.9	0
PAA	90	MAP	4.5	0

ASC, acidified sodium chlorite; CD, chlorine dioxide; MAP, modified atmosphere packaging; PAA, peroxyacetic acid; PB, perforated bag; SH, sodium hypochlorite.

(RZBC, Rizhao, China). Sanitizer's efficiency was compared with SH (Clorox, Santiago, Chile) as conventionally used by the industry (100 mg/L). Electric conductivity and pH were measured with a pH meter (pH 21, Hanna Instruments, Woonsocket, RI) and free Cl_2 was measured with a photometer (HI 95771C, Hanna Instruments) in order to guarantee the effectiveness of chlorination process by maximizing the proportion of hypochlorous acid, the chemical active form to sanitization.

The leaves were pooled to minimize heterogeneity and were immersed for 90 s in different sanitizer solutions (1 kg watercress per 5 L solution) at 5C. Leaves were rinsed in water (5 L/kg) at 5C for 30 s and drained on a stainless steel mesh. The remaining water was removed with a hand centrifuge.

Summer-harvested watercress was treated only using the best dose of each sanitizer based on microbiological and sensory results obtained from the first experiment. Treatments with CD (10 mg/L), ASC (500 mg/L), PAA (90 mg/L) and SH (100 mg/L) were chosen and carried out following the same procedure described above.

Modified Atmosphere Packaging (MAP)

The packaging material was selected according to a permeability model proposed by Artés (1976). Bags (PD-961EZ, Cryovac, Sealed Air Corporation Chile, Santiago, Chile) of 250×150 mm, with permeability values of 7,000 mL/m^2/day for O_2 and 21,000 mL/m^2/day for CO_2 at 23C and 1 atm, were selected.

After the treatment with different sanitizers, leaves (100 g) were packed in plastic bags, which were thermally sealed using a packaging machine (Multivac, Wolfertschwenden, Germany). An extra sample treated with SH was prepared as a control that simulate a humidified air condition (around 20% O_2, ≤0.5% CO_2 and ≥95% relative humidity) in order to compare the effect of different sanitizers on modified atmosphere packaging. This sample was packaged and sealed in a plastic bag with seven perforations made with a 0.7 mm in diameter needle. Five replicates were performed for each treatment stored at 5C for 13 days.

Respiration Rate

The respiration rate was determined as an expression of the metabolic activity of watercress using a static method (Escalona *et al.* 2006). For each treatment, 100 g of samples was placed into 1 L of glass jars and was hermetically closed. Five replicates were performed for each treatment stored for 13 days at 5C. The initial headspace composition (O_2, CO_2 and N_2) was monitored after 1 h by taking a gas sample through an airtight silicone septum on the lid with a 10-mL plastic syringe and injecting it into a gas chromatograph (Hewlett Packard 5890 Series II, Palo Alto, CA) equipped with a thermal conductivity detector. The headspace was analyzed periodically during the storage period. The respiration rate was expressed as CO_2 production (mg/kg/h).

Determination of Gas Composition inside Packages

The evolution of O_2 and CO_2 concentrations expressed as percentage in the headspace of individual packages with and without perforations were determined with the same procedure detailed above. These measurements were conducted at selected time intervals until the end of the storage period.

Microbiological Analysis

The total aerobic mesophilic bacteria, *Enterobacteriaceae*, yeasts and molds were determined. Individual packages were aseptically opened and one sample (10 g) was taken for microbiological analyses. The sample was placed in a sterile plastic bag (Steriblend, Sterilin Limited, Newport, UK) with 90 mL peptone water 0.1% and processed in a stomacher (AES Chemunex, Bruz, France) for 45 s. Serial dilutions were prepared in 9 mL peptone water 0.1% and were plated in the following culture media and incubation conditions: aerobic mesophilic bacteria (plate count agar; 37C for 2 day), yeasts and molds (acidified potato dextrose agar, acidified with lactic acid, pH 3.5, 22C for 5 days). All microbial counts were reported as logarithm of colony-forming units per gram of sample ($\log_{10}$ cfu/g). Five individual bags were analyzed for each treatment, and three serial dilutions from each determination were plated in triplicate. All culture media were purchased from Merck Chemicals (Darmstadt, Germany).

Color Measurements

Color changes on watercress leaves were measured with a tristimulus colorimeter (Minolta CR-300, Ramsey, NJ), 8 mm in diameter of viewing aperture, D_{65} illuminant and 0° observer angle, previously calibrated with color standards in the CIELab system. Color parameters were expressed as lightness (L^*), chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$] and hue angle ($H_{ab} = \tan^{-1} b^*/a^*$). The measurements were performed on 10 leaves per bag, on the adaxial side supporting it on a black surface to prevent color interference, at selected time intervals during cold storage.

Sensory Evaluation

Watercress was assessed using a descriptive–quantitative method by 12 trained judges (Stone and Sidel 2004). Visual

TABLE 2. RESPIRATION RATE (mg CO_2 kg/h) OF SPRING-HARVESTED WATERCRESS TREATED WITH DIFFERENT SANITIZING SOLUTIONS STORED AT 5C

Time (days)	Sanitizer						
	SH	CD		ASC		PAA	
	(100 mg/L)	(5 mg/L)	(10 mg/L)	(250 mg/L)	(500 mg/L)	(50 mg/L)	(90 mg/L)
0	109 B,ab*	118 B,bc	89 B,a	131 B,bc	119 B,bc	135 B,c	125 B,bc
6	62 A,bc	36 A,a	54 A,ab	79 A,c	72 A,bc	59 A,abc	57 A,abc
10	47 A,a	48 A,a	45 A,a	61 A,a	65 A,a	55 A,a	51 A,a
13	50 A,a	47 A,a	46 A,a	62 A,a	58 A,a	45 A,a	40 A,a

* Mean value of five replicates. Values followed by different letters (A–B or a–c) are significantly different ($p < 0.05$). Capital letters compare within a column and lowercase compare within a row.
ASC, acidified sodium chlorite; CD, chlorine dioxide; PAA, peroxyacetic acid; SH, sodium hypochlorite.

appearance, turgidity and off-flavor were evaluated using unstructured patterns of 0–15 cm. Three-digit codified samples were randomly distributed.

Statistical Analysis

The experiment followed a completely randomized design. Data were analyzed by analysis of variance and Tukey's test ($p < 0.05$). MINITAB statistical software, release 13.32 for Windows (Minitab Inc., State College, PA) was used for all statistic tests.

RESULTS AND DISCUSSION

Respiration Rate

At the beginning of the storage period, the respiration rates of spring-harvested watercress were in the range of 89–135 mg CO_2 kg/h and steadily declined in all treatments until reaching constant rates of 45–65 mg CO_2 kg/h after 10 days (Table 2). The high respiration rates observed right after processing could be explained by the physical wounding produced during the preparation process (by washing, rinsing, drying) that caused a subsequent physiological response in the tissue (Klotz *et al.* 2010). It is also known that perishability of vegetables is usually proportional to respiration rate (Kader 2002; Waghmarea *et al.* 2013), which could be intensified by microorganism respiration as reported by Varoquax *et al.* (1996) who observed an increasing respiration rate in minimally processed products with high microbial counts.

Respiration rate of watercress was not modified by different sanitizers at any season (Tables 2 and 3). However, the highest rates were registered at the beginning of conservation on spring-harvested watercress, in samples treated with PAA (50 mg/L) and ASC (250 mg/L), which had respiration values of 135 and 131 mg CO_2 kg/h, respectively. In contrast, initially the lowest respiration rate (89 mg CO_2 kg/h) was obtained for CD (10 mg/L), which decreased to 45 mg CO_2 kg/h after 10 days of storage. This rate was similar to the one obtained using SH. However, it has to be noted that after respiration reached constant values (on day 10), no significant differences ($p < 0.05$) were observed among treatments. A similar trend was reported by Vandekinderen *et al.* (2008), where the modification of respiration rate was also influenced by other factors such as period and temperature of storage, variety of vegetables and processing rather than the oxidative action of the sanitizer.

Initial respiration rates of summer-harvested watercress showed lower values (80–99 mg CO_2 kg/h) than the spring-harvested watercress described above, without significant differences among treatments (Table 3). However, during cold storage, the respiration rates decreased to values similar to those found during the first experiment. The lowest respiration rate was obtained for the PAA treatment (44 mg CO_2 kg/h) and the highest one for SH washing (72 mg CO_2 kg/h) after 13 days of storage.

The observed results coincided with those registered by Vandekinderen *et al.* (2008) and López-Gálvez *et al.* (2009), where no influence of different chemical sanitizers was

TABLE 3. RESPIRATION RATE (mg CO_2 kg/h) OF SUMMER-HARVESTED WATERCRESS TREATED WITH DIFFERENT SANITIZING SOLUTIONS STORED AT 5C

Time (day)	Sanitizer			
	SH (100 mg/L)	CD (10 mg/L)	ASC (500 mg/L)	PAA (90 mg/L)
0	99 B,a*	96 B,a	80 A,a	87 B,a
4	74 A,b	75 AB,b	57 A,ab	48 A,a
7	61 A,a	61 A,a	59 A,a	55 A,a
13	72 A,b	58 A,ab	61 A,ab	44 A,a

* Mean value of five replicates. Values followed by different letters (A–B or a–b) are significantly different ($p < 0.05$). Capital letters compare within a column and lowercase compare within a row.
ASC, acidified sodium chlorite; CD, chlorine dioxide; PAA, peroxyacetic acid; SH, sodium hypochlorite.

TABLE 4. HEADSPACE O_2 AND CO_2 LEVELS (%) IN MAP BAGS STORED AT 5C OF SPRING-HARVESTED WATERCRESS TREATED WITH DIFFERENT SANITIZING SOLUTIONS

Time (days)	HS (100 mg/L)	CD (10 mg/L)	CD (5 mg/L)	ASC (250 mg/L)	ASC (500 mg/L)	PAA (50 mg/L)	PAA (90 mg/L)
				$\%CO_2$			
0	0.70 A,a	0.69 A,a	0.63 A,a	0.95 A,a	0.80 A,a	0.83 A,a	0.99 A,a
1	2.52 B,a	2.69 BC,a	2.56 B,a	2.81 B,a	3.09 B,a	2.53 B,a	2.40 B,a
5	2.66 B,ab	2.84 BC,ab	3.30 BC,bc	3.88 C,c	3.36 B,bc	2.84 B,ab	2.19 B,a
8	2.43 B,a	3.27 C,ab	3.59 C,b	3.46 BC,b	3.72 B,b	3.43 B,b	3.74 C,b
13	2.13 B,a	2.35 B,ab	2.50 B,ab	2.91 B,ab	3.16 B,b	2.53 B,ab	2.53 B,ab
				$\%O_2$			
0	16.58 B,a	17.23 B,a	16.30 C,a	16.90 B,a	16.35 C,a	17.36 B,a	17.65 B,a
1	14.82 B,a	14.53 B,a	14.41 C,a	14.35 B,a	13.56 C,a	14.52 B,a	14.88 B,a
5	8.89 A,a	7.30 A,a	7.04 B,a	5.36 A,a	6.75 B,a	7.08 A,a	7.83 A,a
8	5.68 A,a	5.15 A,a	2.81 A,a	2.21 A,a	2.49 A,a	5.46 A,a	4.03 A,a
13	7.68 A,b	6.13 A,ab	6.07 AB,ab	4.53 A,ab	2.94 AB,a	5.73 A,ab	6.22 A,ab

Mean value of five replicates. Values followed by different letters (A–C or a–c) are significantly different ($p < 0.05$). Capital letters compare within a column and lowercase compare within a row.
ASC, acidified sodium chlorite; CD, chlorine dioxide; PAA, peroxyacetic acid; SH, sodium hypochlorite.

detected over respiration rate and the differences on the respiration rate after processing were adjudicated to the size of the vegetal product and the level of damaged caused by processing. Martínez-Sánchez *et al*. (2008) found that the intensity of the response depends on the type of processing, as observed in leaves treated with cold water that presented a lower respiration rate than those washed with ozonized and hot water (50C).

Modified Atmosphere

The gas concentrations inside the selected plastic bags (Tables 4 and 5) show that perforated bags (PBs) reached an atmosphere condition with 17.6% O_2 and less than 1% CO_2. Oxygen levels decreased and CO_2 levels increased in all MAP packages during the storage period at 5C mainly due to watercress respiration and the gas permeability of the selected plastic film. Oxygen levels of spring-harvested watercress were reduced to a range of 2.2–5.7% after 8 days of storage and the highest O_2 consumption resulting from ASC treatments (Table 4).

Carbon dioxide levels readily increased during the first 24 h (close to 3%), and these levels were maintained during storage. None of the sanitizers applied produced significant modifications over respiration rate so the registered atmosphere were according to consumption and production levels of O_2 and CO_2, respectively. The same patterns were observed at the second experiment developed with summer-harvested watercress (Table 5).

These results coincided with those reported by Allende *et al*. (2008) who studied the effect of seven commercial sanitizers on minimally processed lettuce. They observed that the gas headspace composition within bags treated with ASC (500 mg/L), PAA (80 μL/L) and SH (100 mg/L) was very similar, except for minimally processed salads washed with high concentration of lactic acid (Purac, 20 mL/L), which showed the highest CO_2 accumulation (about 25%) at the end of simulated commercial storage (3 days at 4C and 5 days at 8C). The authors attributed this behavior to an increase in the respiration rate and, as a consequence, a reduction on the shelf life of the product.

TABLE 5. HEADSPACE O_2 AND CO_2 LEVELS (%) IN MAP BAGS STORED AT 5C OF SUMMER-HARVESTED WATERCRESS TREATED WITH DIFFERENT SANITIZING SOLUTIONS

Time (days)	HS (100 mg/L)	CD (10 mg/L)	ASC (500 mg/L)	PAA (90 mg/L)
	$\%CO_2$			
0	0.97 A,a	0.94 A,a	0.73 A,a	0.73 A,a
1	2.14 B,a	2.35 B,a	2.6 B,a	2.70 B,a
5	2.68 BC,a	3.01 BC,a	3.33 B,a	2.92 B,a
8	2.17 B,a	2.71 B,ab	3.37 B,b	3.45 B,b
13	3.14 C,a	3.62 C,a	3.46 B,a	3.17 B,a
		$\%O_2$		
0	16.28 C,a	16.90 C,a	16.21 C,a	16.88 B,a
1	16.19 C,a	15.60 C,a	14.84 C,a	14.01 B,a
5	10.16 B,a	8.31 B,a	6.48 B,a	7.61 A,a
8	5.27 A,a	4.45 AB,a	4.18 AB,a	3.85 A,a
13	4.96 A,a	2.80 A,a	2.28 A,a	4.63 A,a

Mean value of five replicates. Values followed by different letters (A–C or a–b) are significantly different ($p < 0.05$). Capital letters compare within a column and lowercase compare within a row.
ASC, acidified sodium chlorite; CD, chlorine dioxide; PAA, peroxyacetic acid; SH, sodium hypochlorite.

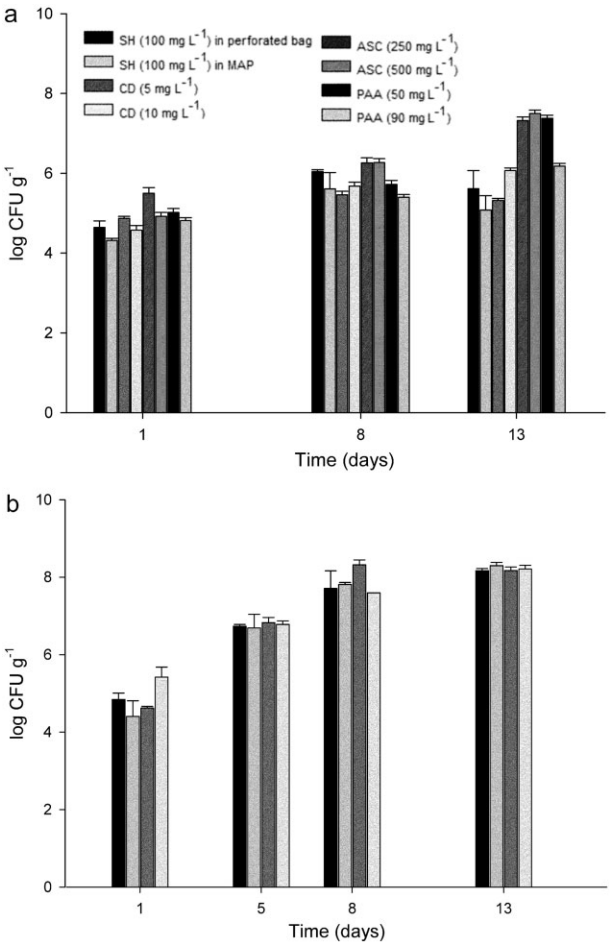

FIG. 1. TOTAL AEROBIC MESOPHILIC BACTERIA IN WATERCRESS AS INFLUENCED BY DIFFERENT SANITIZING TREATMENTS AND STORED IN MODIFIED ATMOSPHERE AT 5C
(a) Spring-harvested watercress; (b) summer-harvested watercress.

Microbial Growth

Total Aerobic Mesophilic Bacteria. Initial count of total mesophilic aerobic bacteria in raw material harvested in spring was 5.6 log cfu/g, whereas the microbial load of summer-harvested watercress was 6.3 log cfu/g (data not shown).

Growth of total aerobic mesophilic microflora during cold storage is shown in Fig. 1. Initial microbial load reductions were observed for most tested sanitizers. In spring-harvested watercress, the greater effect was observed for CD and SH (1.0–1.2 log units), which also maintained low counts (approximately 5 log cfu/g) during 13 days at 5C. The other sanitizers were less effective in delaying microbial growth, reaching more than 7 log cfu/g at the end of storage (Fig. 1a). In summer-harvested watercress, the highest reductions were obtained (1.9, 1.6 and 1.4 log units for CD, ASC and SH, respectively). In summer-harvested watercress, high mesophilic counts (around 8 log cfu/g) were obtained with all treatments after 13 days. Nevertheless, none of the sanitizers was good enough to maintain low counts, exceeding 8 log cfu/g at the end of storage (Fig. 1b). These results show the dependence on the initial microbial load to maintain acceptable counts during cold storage.

Modified atmosphere packaging showed an inhibitory effect on microbial proliferation, as shown by lower counts obtained for SH treatment packed under MAP compared with the use of the same sanitizer but packed in a PB. This behavior may have been caused by low oxygen available for aerobic microorganisms (Phillips 1996).

Other authors report similar reduction levels using these sanitizers. López-Gálvez *et al.* (2010) obtained 1.3 and 1.7 log unit reductions in lettuce using SH (100 mg/L) and CD (3 mg/L). In minimally processed lettuce, the initial mesophilic bacteria reduction was between 0.6 and 1.1 log units after washing using combined sanitizer treatments (peroxyacetic, lactic acid, H_2O_2 and citric acid) (López-Gálvez *et al.* 2013). Martínez-Sánchez *et al.* (2006) observed approximately 1 log cycle reduction for aerobic mesophilic counts in rocket using SH (100 mg/L), ASC (250 mg/L) and PAA (300 mg/L). Allende *et al.* (2009) observed high reduction of aerobic mesophilic bacteria (2.5 log units) in cilantro washed with ASC (500 mg/L), and Vandekinderen *et al.* (2009) reached 1.9 log reductions in shredded carrots washed with PAA (80 mg/L).

Enterobacteriaceae. Raw material showed *Enterobacteriaceae* initial counts of 4.2 log cfu/g on spring and 6.2 log cfu/g on summer (data not shown). In spring-harvested watercress with low initial microbial load, washing with SH, CD (10 mg/L) and PAA (50 and 90 mg/L) reduced initial counts by 1 log unit, while ASC (500 mg/L) caused 0.7 log reduction and little effect was observed with the remaining treatments (Fig. 2a). On the other hand, in the second experiment, most sanitizers achieved 0.8 log unit reduction, except for ASC that caused 1.4 log unit reductions. However, this effectiveness did not last for all storage period, showing no residual effect after application (Fig. 2b).

In spring-harvested watercress, CD (5 and 10 mg/L) and SH slightly inhibited *Enterobacteriaceae* proliferation during 13 days, resulting in less than 5.6 log cfu/g (Fig. 2a). All the other treatments also achieved levels <6.5 log cfu/g during storage. Conversely, when raw material had a high initial *Enterobacteriaceae* load (summer-harvested watercress), none of the sanitizing treatments was good enough to inhibit microbial proliferation and they reached high counts (>7.5 log cfu/g), being acceptable for 5 days at 5C (Fig. 2b).

Molds and Yeasts. Molds and yeasts counts in raw material were 1.8 and 1.7 log cfu/g for each harvesting time,

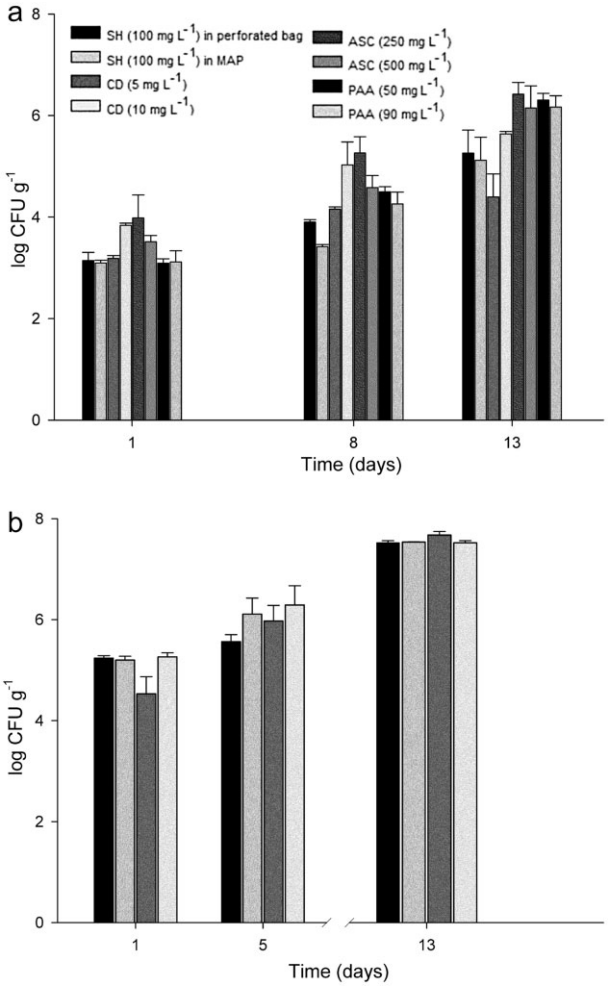

FIG. 2. *ENTEROBACTERIACEAE* IN WATERCRESS AS INFLUENCED BY DIFFERENT SANITIZING TREATMENTS AND STORED IN MODIFIED ATMOSPHERE AT 5C
(a) Spring-harvested watercress; (b) summer-harvested watercress.

respectively (data not shown). The different sanitizers reduced these counts from 0.5 to 0.9 log units. Counts slightly increased during storage and never exceed 2 log cfu/g in all treatments (data not shown). It has to be noted that mold counts were considerably lower than yeast counts.

Slight increases on yeast and mold populations on iceberg lettuce washed with chlorinated water and stored at 5C have been reported (Li *et al*. 2001). These authors also noted that yeast and mold counts were consistently lower (about 3 log cycles) than mesophilic aerobic bacteria.

Sanitizers and modified atmosphere helped to maintain acceptable levels of watercress microbial flora for 8 or 13 days depending on the sanitizer and the initial microbial load of the raw material. CD (10 mg/L) would be a good alternative to replace SH. This sanitizer was effective on diminishing initial microbial load (from 1.0 to 1.9 log units) and also maintained low counts (≤7 log cfu/g) of all native microorganisms in spring-harvested watercress. PAA and ASC also achieved high but tolerable levels of microbial counts under the same conditions. In spite of this, when the raw material had high initial microbial load (summer-harvested samples), all treatments exceeded 8 log cfu/g of mesophilic aerobic microorganisms at the end of storage, leading to a reduction of shelf life to 8 days. López-Gálvez *et al*. (2010) also demonstrated that the use of CD (3 mg/L) was equally effective as SH (100 mg/L), without any detrimental effect on the sensory quality and the bioactive compounds content of minimally processed iceberg lettuce avoiding the formation of trihalomethans. Similar results were reported by Allende *et al*. (2008) who observed a fast increment of mesophilic bacteria in lettuce washed with PAA (80 mg/L) and ASC (500 mg/L), reaching 7.4 log cfu/g after 8 days of storage.

The efficiency of the sanitizers could be related to both high oxidizing capability and high surfactant activity, which allows better contact between attached bacteria and the active compound of the sanitizers (Sapers 2001). Beuchat and Brackett (1990) found that SH washings reduced initial bacteria counts in lettuce, but after 4 days at 5C no significant differences were observed with SH or water washings.

Allende *et al*. (2004) reported that in many steps of the process the bacterial counts of minimally processed vegetables increased, being the washing a key step to reduce the microbial load. The recycled washing water could act as a vehicle for vegetable cross-contamination. In this sense, alternative sanitizers can prevent this undesirable process by inactivating most of the microorganisms removed from vegetable surfaces and could be an interesting alternative to SH (Zhang *et al*. 2009).

Microbial load of watercress is generally higher than other vegetables because the leaves are exposed and are not protected as inner leaves, i.e., lettuce heads. This was also reported by Allende *et al*. (2008), where the initial microbial load for escarole was higher than iceberg lettuce (6.9 and 3.6 log cfu/g for mesophilic bacteria and 5.4 and 2.4 log cfu/g for coliforms, respectively).

Seasonal variation of microbial load and visual quality of commercial minimally processed baby leaf salads (lettuce, rockets, spinach, lamb's lettuce) was reported by Caponigro *et al*. (2010). They observed that samples of summer and autumn months showed significantly higher counts for all microbiological parameters and lower quality scores compared with winter and spring months. Total mesophilic aerobic counts at the consume by date were above 8 log cfu/g for 18% of the samples with visual quality declination. They stated that the relationship between microbial load and visual quality was statistically significant for total mesophilic aerobic bacteria, coliforms and lactic acid bacteria. This study is in agreement with our findings,

TABLE 6. COLOR PARAMETERS OF SPRING-HARVESTED WATERCRESS TREATED WITH DIFFERENT SANITIZING SOLUTIONS AND STORED IN MODIFIED ATMOSPHERE AT 5C

		Sanitizer							
	Time	SH		CD		ASC		PAA	
Parameter	(day)	(100 mg/L)*	(100 mg/L)	(10 mg/L)	(5 mg/L)	(250 mg/L)	(500 mg/L)	(50 mg/L)	(90 mg/L)
L^*	1	45.9 A,a†	44.4 A,a	47.6 AB,a	45.0 A,a	46.1 A,a	45.8 A,a	44.4 A,a	45.2 A,a
	5	48.8 A,a	45.6 A,a	46.8 A,a	49.2 B,a	49.2 B,a	49.2 B,a	47.4 A,a	47.7 A,a
	8	53.1 B,a	50.7 B,a	50.1 B,a	51.8 B,a	53.5 C,a	52.8 C,a	50.8 B,a	52.1 B,a
	13	60.7 C,c	55.1 C,a	55.9 C,ab	58.2 C,abc	59.3 D,bc	57.5 D,abc	57.4 C,abc	56.5 C,ab
C^*	1	33.8 A,ab	31.9 A,a	35.2 A,b	33.1 A,ab	34.5 A,ab	33.6 A,ab	32.7 A,ab	33.3 A,ab
	5	36.2 B,bc	32.9 A,a	34.5 A,ab	37.1 B,bc	37.4 B,c	36.4 B,bc	35.7 B,abc	35.2 A,abc
	8	40.6 C,a	38.9 B,a	38.9 B,a	40.5 C,a	41.6 C,a	41.2 C,a	38.9 C,a	40.3 B,a
	13	44.5 D,b	41.2 B,a	43.6 C,ab	45.4 D,b	46.3 D,b	43.8 D,ab	43.8 D,ab	43.5 C,ab
H_{ab}	1	125.7 C,a	126.5 C,a	124.3 C,a	125.6 C,a	125.0 C,a	125.5 D,a	126.3 C,a	125.8 C,a
	5	124.1 C,ab	125.9 C,b	125.0 BC,ab	123.4 BC,ab	122.9 C,a	123.3 C,ab	124.3 BC,ab	124.3 C,ab
	8	121.7 B,a	122.5 B,a	122.5 B,a	121.6 B,a	120.3 B,a	120.6 B,a	122.4 B,a	121.6 B,a
	13	115.9 A,ab	120.2 A,c	118.5 A,bc	116.3 A,b	114.6 A,a	116.7 A,ab	117.6 A,bc	118.6 A,bc

* Packed in a perforated bag. All the other samples were packed under passive modified atmosphere (MAP).

† Mean value of five replicates. Values followed by different letters (A–D or a–c) are significantly different ($p < 0.05$). Capital letters compare within a column and lowercase compare within a row.

ASC, acidified sodium chlorite; CD, chlorine dioxide; PAA, peroxyacetic acid; SH, sodium hypochlorite.

except for our observation that the rate of increment of microbial populations was affected by the level of initial contamination.

Color

Lightness (L^*) increased in leaves treated with all sanitizers and MAP during cold storage (Tables 6 and 7), indicating chlorophyll degradation. Chroma (C^*) values increased and hue angle diminished turning from a bluish-green to a yellowish area during storage. None of the sanitizer prevented color losses, and a slight effect of MAP could be detected in samples packaged on air (PB) that showed increment of lightness and C^* values at the end of storage.

At the beginning of the first experiment (spring-harvested watercress), no significant differences were observed among treatments (Table 6). Color differences became more evident at the end of the storage, where the highest discoloration was observed for ASC treatments. On the other hand, watercress better kept its color when treated

TABLE 7. COLOR PARAMETERS OF SUMMER-HARVESTED WATERCRESS LEAVES TREATED WITH DIFFERENT SANITIZING SOLUTIONS AND STORED IN MODIFIED ATMOSPHERE AT 5C

		Sanitizer			
Parameter	Time (day)	SH (100 mg/L)	CD (10 mg/L)	ASC (500 mg/L)	PAA (90 mg/L)
L^*	1	44.1 A,a*	43.6 A,a	44.7 A,a	44.8 A,a
	5	45.2 A,a	45.4 B,a	45.5 A,a	45.4 AB,a
	8	44.9 A,a	46.7 B,b	45.4 A,ab	46.4 B,b
	13	48.8 B,a	51.0 C,c	49.5 B,ab	50.7 C,bc
C^*	1	27.5 A,a	26.6 A,a	26.9 A,a	26.5 A,a
	5	30.7 B,a	30.2 B,a	31.0 B,a	30.2 B,a
	8	30.4 B,a	32.4 B,a	31.4 B,a	31.3 B,a
	13	34.4 C,a	36.7 C,a	35.2 C,a	36.6 C,a
H_{ab}	1	126.2 B,a	126.3 B,a	126.8 C,a	125.9 B,a
	5	125.9 B,a	126.8 B,a	125.9 BC,a	127.0 B,a
	8	125.2 B,ab	126.5 B,b	124.4 AB,a	126.4 B,b
	13	123.3 A,b	120.7 A,a	123.1 A,b	121.7 A,ab

* Mean value of five replicates. Values followed by different letters (A–C or a–c) are significantly different ($p < 0.05$). Capital letters compare within a column and lowercase compare within a row.

ASC, acidified sodium chlorite; CD, chlorine dioxide; PAA, peroxyacetic acid; SH, sodium hypochlorite.

with SH, CD (10 mg/L) and PAA (90 mg/L) and packed in modified atmosphere without significant differences. In the second experiment (summer-harvested watercress), the color parameters did not show changes until day 8 where L^* and C^* values increased and lower H_{ab} values were registered compared with day 1 (Table 7).

Sensory Quality

Visual appearance started to decrease after 5 days of storage in both spring- and summer-harvested watercress (Tables 8 and 9). However, all the treatments remained above acceptable scores until 8 days of storage. Only SH and ASC (500 mg/L) had acceptable scores after 13 days. For summer-harvested watercress, the best treatments were PAA and ASC. The worst evaluated treatment was CD with a low appearance score at the end of storage in both experiments.

Significant differences ($p < 0.05$) on color intensity were evident on day 8 with a notorious decrease of the scores, and CD washing resulted hardly acceptable (Table 8). In the second experiment, the intensity of color remained with high scores for longer time: watercress washed with PAA and ASC resulted acceptable for 13 days (Table 9). In general, these results agree with the colorimetric measures.

Modified atmosphere had a clear beneficial effect on preserving green color. The samples treated with SH packed in PB were unacceptable after 8 days of storage and received the lowest scores at any time compared with SH in MAP. These results are in agreement with those reported by Oms-Oliu *et al.* (2009), who showed that low O_2 and moderate CO_2 atmospheres combined with low storage temperatures and high relative humidity delays yellowing in leafy vegetables. Also a significant delay in greening and browning of endive was observed using a reduced oxygen level MAP (Charles *et al.* 2008).

Turgidity was not affected by the type of sanitizer, but it was affected by storage conditions and time (Tables 8 and 9). The important role of MAP preserving this attribute was shown by the lowest scores for the same SH treatment in PB. The other treatments were acceptable at that time.

The scores assigned to off-flavor for both types of watercress were all acceptable during storage (Tables 8 and 9). A trend to increase with time was observed because judges found that natural spicy taste of watercress was stronger. However, this may be due to elapsed time instead to the treatment with sanitizers itself.

The CD treatment negatively affected sensory quality of watercress stored at 5C. However, from the point of view of microbiological safety, CD (10 mg/L) could be a good alternative to replace SH. Further studies should be performed in order to take advantage of the antimicrobial effectiveness of CD preventing its detrimental effect on sensory quality, using it in a low concentration in combination with another sanitizer or preservation technology.

TABLE 8. SENSORY PARAMETERS OF SPRING-HARVESTED WATERCRESS TREATED WITH DIFFERENT SANITIZING SOLUTIONS AND STORED IN MODIFIED ATMOSPHERE AT 5C

		Sanitizer							
	Time	SH		CD		ASC		PAA	
	(day)	(100 mg/L)*	(100 mg/L)	(10 mgL)	(5 mg/L)	(250 mg/L)	(500 mg/L)	(50 mg/L)	(90 mg/L)
Visual appearance	1	11.0 B,a†	11.4 AB,a	11.0 B,a	10.8 B,a	11.4 B,a	13.0 C,a	11.9 B,a	11.8 BC,a
	5	11.0 B,a	12.1 B,a	11.0 B,a	10.7 B,a	11.4 B,a	11.5 BC,a	11.2 B,a	12.5 C,a
	8	8.7 AB,a	9.8 AB,a	9.1 B,a	8.8 AB,a	9.2 B,a	9.5 AB,a	9.6 AB,a	9.5 AB,a
	13	8.0 A,bc	9.0 A,c	5.6 A,ab	4.1 A,a	6.2 A,abc	8.5 A,bc	7.4 A,bc	7.1 A,abc
Color intensity	1	10.8 C,a	10.5 B,a	11.4 C,a	11.0 C,a	11.6 C,a	11.8 C,a	11.2 B,a	11.9 C,a
	5	10.2 BC,a	11.0 B,a	10.2 C,a	10.7 C,a	10.9 C,a	10.6 BC,a	10.8 B,a	11.3 C,a
	8	7.6 AB,a	9.5 AB,a	7.2 B,a	7.4 B,a	8.0 B,a	8.8 AB,a	9.3 B,a	8.2 B,a
	13	5.1 A,bcd	7.1 A,cd	2.5 A,ab	1.7 A,a	4.0 A,abc	7.6 A,d	6.2 A,cd	4.8 A,abcd
Turgidity	1	8.4 A,a	9.5 A,a	9.5 A,a	9.5 A,a	10.2 B,a	9.4 A,a	10.0 A,a	10.7 A,a
	5	7.8 A,a	8.7 A,a	9.0 A,a	7.4 A,a	10.4 B,a	9.8 A,a	10.0 A,a	9.7 A,a
	8	7.2 A,a	7.7 A,a	8.7 A,a	8.7 A,a	7.6 AB,a	7.5 A,a	7.8 A,a	8.9 A,a
	13	6.9 A,a	7.7 A,a	7.7 A,a	7.3 A,a	6.9 A,a	7.4 A,a	6.9 A,a	8.2 A,a
Off-flavor	1	2.0 A,a	1.1 A,a	2.4 A,a	1.5 A,a	2.2 A,a	1.3 A,a	1.4 A,a	2.3 A,a
	5	2.7 A,a	2.5 A,a	3.9 A,a	2.9 A,a	3.4 A,a	2.5 A,a	2.8 A,a	4.3 A,a
	8	4.3 A,a	3.9 A,a	3.8 A,a	4.2 A,a	3.5 A,a	3.7 A,a	1.8 A,a	5.3 A,a
	13	1.8 A,a	2.1 A,a	2.0 A,a	2.3 A,a	2.9 A,a	3.3 A,a	3.0 A,a	4.0 A,a

* Packed in a perforated bag. All the other samples were packed under modified atmosphere (MAP).

† Mean value of five replicates. Values followed by different letters (A–C or a–d) are significantly different ($p < 0.05$). Capital letters compare within a column and lowercase compare within a row.

ASC, acidified sodium chlorite; CD, chlorine dioxide; PAA, peroxyacetic acid; SH, sodium hypochlorite.

TABLE 9. SENSORY PARAMETERS OF SUMMER-HARVESTED WATERCRESS TREATED WITH DIFFERENT SANITIZING SOLUTIONS AND STORED IN MODIFIED ATMOSPHERE AT 5C

		Sanitizer			
	Time (days)	SH (100 mg/L)*	CD (10 mg/L)	ASC (500 mg/L)	PAA (90 mg/L)
Visual appearance	1	12.43 B,a†	11.88 B,a	12.08 B,a	11.42 B,a
	4	11.50 B,a	10.50 B,a	11.63 B,a	11.34 B,a
	7	10.51 B,a	10.04 B,a	10.00 AB,a	10.60 AB,a
	13	7.06 A,b	4.76 A,a	7.97 A,b	8.50 A,b
Color intensity	1	11.08 B,a	11.21 B,a	11.40 B,a	11.52 A,a
	4	11.75 B,a	10.42 B,a	11.14 B,a	11.74 A,a
	7	10.78 B,a	10.03 B,a	10.72 B,a	10.81 A,a
	13	7.22 A,b	4.82 A,a	8.55 A,bc	9.74 A,c
Turgidity	1	11.15 B,a	10.50 B,a	11.64 B,a	10.49 A,a
	4	8.55 A,a	9.03 AB,a	10.01 AB,a	9.21 A,a
	7	9.69 AB,a	9.48 AB,a	10.44 AB,a	9.98 A,a
	13	7.90 A,a	7.88 A,a	8.17 A,a	8.89 A,a
Off-flavor	1	4.49 A,a	2.69 A,a	2.81 A,a	2.94 A,a
	4	4.07 A,a	3.81 A,a	3.88 A,a	3.90 A,a
	7	3.35 A,a	4.21 A,a	4.86 A,a	4.64 A,a
	13	4.56 A,a	5.65 A,a	5.47 A,a	4.23 A,a

* Packed in a perforated bag. All the other samples were packed under modified atmosphere (MAP).

† Mean value of three replicates. Values followed by different letters (A–B or a–c) are significantly different ($p < 0.05$). Capital letters compare within a column. Lowercase compare within a row. ASC, acidified sodium chlorite; CD, chlorine dioxide; PAA, peroxyacetic acid; SH, sodium hypochlorite.

CONCLUSIONS

All tested sanitizers combined with modified atmosphere helped to maintain acceptable levels of microbial flora of watercress for 5–8 days depending on the sanitizer and the initial microbial load of the raw material. CD (10 mg/L) was the most effective sanitizer on diminishing initial microbial load and also maintained low counts of native microorganisms during 13 days at 5C. PAA and ASC achieved a high but tolerable level of microbial counts under the same conditions. Therefore, all tested sanitizers could be used to replace SH. In spite of this, if the raw material had a high initial microbial load (summer-harvested watercress), the alternative sanitizers only caused a high initial reduction of this load and could not properly manage the spoilage during the remaining storage period.

The type of sanitizer did not affect the sensory characteristics of watercress right after processing. However, differences on visual appearance and sensory color intensity were evident at the end of storage. MAP contributed to diminish tissue respiration, to inhibit microbial proliferation and to delay color deterioration in leafy vegetables.

The use of sanitizers reduced initial counts near to 2 log units, but could not handle high microbial loads. It showed the need of implementing good agricultural practices to produce high-quality raw material. Therefore, the preparation of minimally processed vegetables required the use of raw material with high microbiological quality in order to obtain a safe product with extended shelf life.

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